PLANT BIOTECHNOLOGY IN CHINA

TRIP REPORT
September 16-30, 1993

Alvin L. Young
Sue A. Tolin
J. Neil Rutger
Donald D. Kaufman
Martha Bair Steinbock

Prepared for:

Research and Scientific Exchange Division
Office of International Cooperation and Development
United States Department of Agriculture
Washington, DC

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EXECUTIVE SUMMARY

A team of five U.S. Biotechnology specialists visited China, September 16-30, 1993 as part of the U.S. - China Scientific Exchange Program. **The purpose of the visit was to survey and assess the status of Chinese plant biotechnology research programs.** The team visited 13 locations in four cities (Beijing, Nanjing, Wuhan, Guangzhou).

In 1986, China launched a major effort to develop biotechnology research to enhance its economic development and solve major problems facing its agricultural and industrial sectors. Given the importance of agriculture to the Chinese economy, and need to feed an expanding population, agricultural biotechnology research receives a relatively large share of China's overall biotechnology research budget. Biotechnology research is funded through a well organized network of institutions and mechanisms to achieve goals set out in the current Five Year Plan (1991 - 1995). National coordination is provided by the China National Center for Biotechnology Development (CNCBD).

Major strategies which involve applications of molecular biology to plants include: transforming plants (cotton, corn, poplar, etc.) with the *Bacillus thuringensis* (Bt) gene for insect resistance; engineering vegetables, fruits, legumes, rice and tobacco for virus resistance; improving the yields and quality of rice through creating two line hybrids; and engineering rhizobia to enhance nitrogen fixation.

China's greatest strength in the future development of agricultural biotechnology will be its human resources. The team was not only impressed with the large number of undergraduate and graduate students interested in agricultural biotechnology, but also with the spirit of innovation and enthusiasm with which they approached their work. China is doing some unique agricultural biotechnology research which is relatively unknown by the international scientific community.

International contacts will be key to the development of agricultural biotechnology in China. All the outstanding Chinese research institutions have long-standing relationships with research institutions in the United States or Europe. These contacts have allowed leading Chinese biotechnology researchers access to the latest scientific information as well as access to facilities for performing types of research which are difficult to carry out in China, due to the poor infrastructure. **Japan and the European Community are assisting China in developing biotechnology in order to forge scientific and commercial linkages and be in on the "ground floor" of Chinese biotechnology development.**

China currently does not have formal national oversight procedures for reviewing the safety of field tests of genetically modified plants, animals or microbes. Guidelines have been drafted by an expert committee reporting to the CNCBD and are awaiting State approval.

The Chinese Government also recognizes the importance of improving its protection of intellectual property. **China wishes to be an active participant in the projected international biotechnology market place, and recognizes that protection of intellectual property is a fundamental prerequisite of such participation.**

China had conducted many small-scale field tests of genetically modified plants and associated microorganisms. Among the species reported or observed by the team in the field were rice, maize, wheat, cotton, barley, soybeans, peanuts, alfalfa, grapes, tomatoes, tobacco, and an extensive array of vegetable

crops. **Two genetically engineered organisms were reported to be at the stage of full-scale performance trials -- virus resistant tobacco and an improved nitrogen-fixing rhizobium.** The transgenic tobacco, which was tested on 10,000 hectares in 1992, was harvested and processed for cigarettes. The soybean rhizobium was tested on over 30,000 hectares. The soybeans from the test plots were consumed.

If China is successful in applying the techniques of modern biology to their agricultural sector, this will undoubtedly have an impact on U.S. - China agricultural trade. China's tobacco, cotton, maize, rice, oilseed, and vegetable production could be augmented by applying biotechnology in the medium-term future. Wheat trade is unlikely to be impacted by biotechnology.

The United States should increase its interactions with China in agricultural biotechnology as part of an overall strategy to create scientific and market opportunities. This strategy should encompass: (1) increased consultation on biosafety and intellectual property rights issues, (2) increased collaboration in selected scientific and technical areas, (3) closer consultations on biotechnology policy in order to enhance the broader relationship between U.S. and Chinese agricultural communities, and (4) exploring ways to assist the U.S. agricultural biotechnology industry in gaining knowledge about opportunities for joint ventures in China.

TRIP REPORT

U.S. Team Visit to China on Molecular Biology Applications to Crop Agriculture

Introduction

A team of U.S. biotechnology experts visited China, September 16-30, 1993, as part of the U.S. - P.R.C. Scientific Exchange Program administered by the Office of International Cooperation and Development of the U.S. Department of Agriculture (USDA) and the Department of International Cooperation of the Ministry of Agriculture of the People's Republic of China. The purpose of the trip was to survey Chinese plant biotechnology research.

The team was composed of five individuals with diverse disciplinary expertise:
Alvin L. Young, Director, USDA/Office of Agricultural Biotechnology (OAB), (team leader); J. Neil Rutger, Director, National Rice Germplasm Research Center, USDA/Agricultural Research Service (ARS) (genetics); Sue A. Tolin, Professor, Department of Plant Physiology, Pathology and Weed Science, Virginia Polytechnic and State University (VPI&SU) (plant pathology); Donald D. Kaufman, Senior Scientist, USDA/ARS, Rodale Institute Research Center, (microbiology); and Martha B. Steinbock, International Affairs Specialist, USDA/OAB (economics).

The team visited 13 locations in four cities (Beijing, Nanjing, Wuhan, Guangzhou), which were selected because of their prominence in published scientific literature about plant biotechnology. The team requested a visit to Hainan Island to see large-scale field testing of genetically modified plants, but this was not included in the itinerary prepared by the Ministry of Agriculture. The team's itinerary is attached as appendix A.

Funding Agricultural Biotechnology Research

In 1986, China launched a major effort to develop biotechnology research to enhance its economic development and solve major problems facing its agricultural and industrial sectors. This biotechnology initiative has three main priorities: agricultural biotechnology, medicine, and protein engineering. Given the importance of agriculture to the Chinese economy, and need to feed an expanding population, agricultural biotechnology research receives a relatively large share of China's overall biotechnology research budget.

The team requested, but did not receive, overall budget data for national agricultural biotechnology research programs. The reason given for not providing these data was that funding is provided by so many different mechanisms, that the budgets of individual agencies are misleading. The types of funding mentioned were: project-based funding awarded competitively on the basis of national solicitations of proposals (covers research costs exclusive of salaries); salaries and living expenses of researchers provided by institutes and universities; special grants for facilities and equipment which are allocated on the basis of national priorities; funding provided by provinces for research programs related to local needs; and funding obtained from sales of commercial products produced by research institutes such as biocontrol agents or improved varieties.

Project-based funding for agricultural biotechnology research in China is provided and administered through a well-established, complex network of agencies and Commissions at the national and provincial levels. Nationally, the three major funding organizations are the High Technology Program of the State Science and Technology Commission (SSTC), the State Commission of Planning, and the Ministry of Agriculture. The Ministry of

Agriculture is the smallest of the three, in terms of the amount of funding provided (12-15% of the total biotechnology budget).

The size of grants varies, with large nationally-funded projects averaging about 200,000 Yuan[1] annually, and smaller projects often funded at about the 50,000 Yuan level. Some projects are as small as 10,000 Yuan per year. Grants from these programs are used for direct research costs, since in most cases, overhead and salaries are fully funded by the research institution. Project commitments are made for five years based on the outcome of interim evaluations of the project. Projects fit objectives and goals established by the national five year plan. The five year project cycle allows researchers ample time to complete key segments of research; however, it also constrains the flexibility of the Government in resetting research goals.

National programs which provide funding for agricultural biotechnology research include: (1) the Torch program which is aimed at technology transfer (funded by development banks): (2) the 863 program aimed at stimulating economically-viable discoveries in key sectors (funded by the State Commission of Planning); (3) applied projects aimed at yield improvements and quality enhancement (funded by the Ministry of Agriculture and the National Center for Biotechnology Development (CNCBD); (4) and to a lesser extent, basic research aimed improving general understanding (funded by the Natural Science Foundation). There is also an impressive investment in teaching and post graduate training, both within Chinese universities and in sending the most capable students and senior scientists abroad.

The overall impression of the team is that there are a small number of key laboratories, which because of their excellence, compete well for grants and receive special funding for facilities and equipment. These laboratories garner a large proportion of the biotechnology budget. On the other hand, although some effort is made to distribute funding evenly, the majority of other laboratories do not receive adequate funds to provide the basic equipment, chemicals and support services necessary to perform state-of-the-art biotechnology research.

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[1] 5.7 Yuan currently equals $1.00 U.S.

The Structure of Research

Agricultural biotechnology research in China is well-organized and is being developed to reach a clear set of national objectives. National coordination is provided by the China National Center for Biotechnology Development (CNCBD), which is administered by the SSTC. The CNCBD funds about 100 projects annually. Many of these are cross-cutting initiatives which exceed the mandate of a single Ministry.

Agricultural biotechnology research is carried out at research institutes affiliated with the Chinese Academy of Science (CAS), institutes affiliated with Chinese Academy of Agricultural Sciences (CAAS), agricultural universities, and State and provincial general universities. Appendix B illustrates the relationships between the various entities which administer and carry out biotechnology research in China.

In addition to the institutes of CAS and CAAS, there are six national laboratories funded largely by the State Commission of Planning, each with a technical specialty. The national laboratory working on plant biotechnology is located at the Huazhong Agricultural University in Wuhan. In addition, there are sixteen universities receiving funding from the Ministry of Agriculture for biotechnology research, including eight key universities.

Both the Ministry of Agriculture and the CNCBD make use of advisory committees composed of scientists selected on the basis of their scientific credentials. These committees serve as peer panels for reviewing proposals, selecting grant recipients, and conducting project evaluations. They also provide advice to the Government concerning issues such as biosafety oversight and research priorities.

Research Priorities

Given the growing population and the losses of agricultural land to industrial development, it is clear that China must keep increased productivity as the top priority for crop agriculture for the foreseeable future. Although there is little visible hunger in most parts of China, currently, the average Chinese family spends 70 percent of their income on food. Thus, there is a dramatic need to produce food more efficiently and to pass on savings to the consumer so that living standards can rise and demand can be created for other segments of the economy. In addition there is quickly a growing demand for higher quality and a greater diversity in agricultural production to meet the needs of foreigners in China, Chinese citizens who earn higher salaries, and for export.

The Five Year Plan (1991-1995) of China identifies a number of techniques as priority areas for agricultural research. These include genetic engineering of plants and animals, micropropagation, embryo transfer, *in vitro* fertilization, recombinant vaccine development, and monoclonal antibody development. These techniques are to be applied to increase yields and to improve the quality of agricultural products.

Major strategies which involve applications of molecular biology to plants include: transforming plants (cotton, corn, poplar, etc.) with the *Bacillus thuringensis* (Bt) gene for insect resistance; engineering vegetables, fruits, legumes, rice and tobacco for virus resistance; improving the yields and quality of rice through creating two line hybrids; engineering rhizobia to enhance nitrogen fixation; mapping genes in populations of rice, barley and wheat; and map-based cloning of genes.

Among the smaller research projects described were: transfer of anti-bacterial peptides to potato, tobacco and rice; improving the protein quality and productivity of plants by increasing the content of sulphur; regulation of gene expression in *Brassica*; research on cytoplasmic male sterility in corn; engineering grapevine for virus

resistance,: control of fruit ripening through research on the ACC synthase pathway; using plants as bioreactors to produce human immunogens, seeking mechanisms for apomixis in rice; and transfer of genes from Chinese medicinal plants to tobacco, etc. Detailed reports of the research at each of the laboratories visited is contained in appendix C.

Assessment of Research Programs

The excellent laboratories visited by the team were the Institute of Microbiology of the Chinese Academy of Sciences in Beijing and the Huazhong Agricultural University in Wuhan. Although the team was not able to visit Beijing University and Shanghai, literature and anecdotal evidence indicates that the Department of Genetics and the Department of Plant Biology at Beijing University and the CAS and University of Shanghai in Shanghai are also capable of sophisticated molecular biology research.

Other institutes have been able to overcome some of their physical limitations through forging research alliances with the leading Chinese research establishments. For example, several of the laboratories visited indicated that they were working with transgenic plants which had been transformed by the Department of Genetics at Beijing University. Likewise, some researchers reported receiving constructs or probes from other laboratories because they did not have the physical infrastructure to produce them in their own laboratories.

Many of the laboratories visited had extensive activity in plant tissue culture. They appeared quite successful with many species of crop, horticultural, medicinal and forest species. New lines had been developed via somoclonal variation and by wide hybridization. Elegant cytogenetic studies characterized chromosomes in these hybrid plants. Molecular markers for mapping genes and analyzing genetic diversity were quite advanced for maize, rice, and wheat.

China's greatest strength in the future development of agricultural biotechnology will be its human resources. An impressive system of over 50 agricultural universities is generating a large cadre of bright young researchers, familiar with at least some of the techniques of molecular biology. The most outstanding students are routinely sent abroad for advanced training.

The team was not only impressed with the large number of undergraduate and graduate students interested in agricultural biotechnology, but also with the spirit of innovation and enthusiasm with which they approached their work. Although these young scientists were very eager to have the opportunity to travel abroad for training, they did not wish only to participate in research programs directed by others. They expressed the desire to do original work. Some of the approaches and techniques discussed during the laboratory visits are unique to China.

Examples of Unique Research

China is conducting pioneering research in a number of areas. A few examples observed by the team include the program of Wang Guo-Ying at Beijing Agricultural University who discussed ultra-sonification method of maize callus transformation. This process apparently breaks the cell walls of callus, permitting DNA uptake and does not rely on protoplast culture. He has published only an abstract so far; but reportedly has herbicide-resistant transgenic maize in the third generation.

Ni Wanchao of the Institute of Industrial Crops at Jiangsu Academy of Agricultural Sciences in Nanjing described a program on cotton biotechnology in which he obtained transformants with DNA of wild cotton injected into the pollen tube in field grown plants. Insect-resistant plants, now in the fifth generation in the field, had a Bt toxin or protease inhibitor gene. He stated that there was no yield reduction or insect resistance, as yet. Scientists in several Chinese laboratories are trying to develop apomixis (asexual seed production) in rice. With apomixis it would be possible produce true-breeding F1 hybrid rice, and farmers would be able to save their own seeds for hybrids. Hybrid rice is reported to yield 15-20% more than conventional rice.

Among the laboratories visited by the team, Mou Tong Min's biotechnology laboratory at the Hubei Academy of Agricultural Science in Wuhan is seeking apomixis through somoclonal mutation, and protoplast fusion with apomictic *Panicum* and *Pennisetum* species. At Huazhong Agricultural University, Cai Detian claims to have induced partial apomixis through irradiation with high energy ions. At the South China Institute of Botany in Guangzhou, Li Yuan Ching suspects there is apomixis in a rice line that sets seed before flowering.

The team also heard about apomixis research in three institutes which it did not visit. Chen Jiansan of the Chinese Academy of Agricultural Science in Beijing has been reporting the presence of apomixis in rice material "84-15" for several years. Scientists at Sichuan Agricultural University are also reporting apomixis in rice. The Hunan Hybrid Rice Research Center in Changsha is studying high frequency twin seedlings as a possible indication of apomixis.

The tobacco breeding program at Nanjing Agricultural University, described by Huqu Zhai, is using wide crosses to transfer useful genes from Chinese herbs into tobacco. The project is now using *Gymnostemma pentophyllum*, which contains an as yet unidentified compound used for heart ailments and antibody stimulation. Equipment has been installed recently to aid in the purification of this compound. The work was presented at the International Genetics Congress in Scotland in August 1993 and received wide attention.

Scientists in the Microbiology Department of Huazhong Agricultural University in Wuhan reported success in placing multiple nitrogen fixation genes from several organisms into a single recipient organism. Although the construct is not stable beyond a single growing season, it is readily reproducible in the laboratory for use as an inoculant in subsequent years. Treatment with the inoculant reportedly has lead to significant increases in yields in soybeans in field tests.

The Role of Linkages with Foreign Laboratories

International contacts are key to the development of agricultural biotechnology in China. All the outstanding Chinese research institutions have established one or more, long-standing relationships with research institutions in the United States, Europe, Australia or New Zealand. Leading Chinese researchers have been able to continue contacts with foreign laboratories over a number of years, often traveling outside China on a regular basis. These contacts have allowed leading Chinese biotechnology researchers access to the latest scientific information as well as access to facilities for performing types of research.

Every laboratory visited seemed eager for more contact with research institutions in the United States. This openness also extends to commercial ventures. Several laboratories visited were actively seeking joint venture partners to provide capital to produce agricultural products in China and for export. For example, the Jiangsu Provincial Research Institute of Forestry Science, near Nanjing, is seeking a joint venture partner for scale-up of

their tissue culture production of horticultural products and forest trees. Some laboratories such as the biotechnology center at Huazhong Agricultural University in Wuhan are officially designated as "open laboratories" which means that their facilities may be used by visiting scientists from within or outside China.

The European Community (EC) and Japan are cultivating long-term research alliances with China. In 1991 the EC and China inaugurated the China - EC Biotechnology Center (CEBC). The CEBC is an administrative entity which coordinates and promotes linkages between Chinese and European laboratories working on agricultural and medical biotechnology. The CEBC maintains an office and a conference room at the headquarters of the CNCBD in Beijing. Each year the EC awards about 15 fellowships to Chinese researchers to allow them to work in European laboratories.

The team also observed a training workshop/short course on molecular biology of Bt being taught by Japanese scientist in Wuhan. Chinese scientists frequently mentioned visiting Japanese laboratories for advanced training.

The Rockefeller Foundation is also playing a key role in promoting applications of agricultural biotechnology in rice research in China. The Foundation provides funding for key research on disease resistance in rice as well as for Chinese participation in the international rice genome project. Within China, the rice genome project is funded by SSTC. Work is being carried out at a number of centers including Shanghai National Academy of Sciences, Beijing University/Institute of Genetics, Huazhong Agricultural University, the China National Rice Research Institute in Hangzhou (CNNRI) and Fudan University.

Other international entities providing funding for biotechnology work in China include the United Nations Industrial Development Organization (UNIDO) and the Food and Agricultural Organization (FAO) of the United Nations. The CNCBD coordinates interactions with international foundations and organizations.

The team also learned that several U.S. agricultural biotechnology companies had been to China to review research and discuss possible collaborative relationships. Representatives of Monsanto have held discussions with policy makers and visited the CAAS Biotechnology Research Center in Beijing and the Jiangsu Academy of Agricultural Sciences in Nanjing. Ecogen has visited the Microbiology Department at Huazhong Agricultural University. If the issue of intellectual property protection is resolved, there is clearly is an abundance of opportunities in China for companies which are willing to make a long-term investment, in order to develop market opportunities in agricultural biotechnology.

The Biosafety Issue

China currently does not have formal national oversight procedures for reviewing the safety of field tests of genetically modified plants, animals or microbes. Guidelines have been drafted by an expert committee reporting to the CNCBD and are awaiting State approval. Representatives of the CNCBD reported to the team that they had studied a number of different "models" including U.S. Government regulations.

The Ministry of Agriculture reported that once the general guidelines are finalized, they will quickly issue specific guidelines for different aspects of agricultural biotechnology such as plants and microorganisms. The Ministry of Agriculture also reported that once the Guidelines are in place, commercialization of new varieties produced with genetic engineering will require two approvals in China -- one under the biosafety guidelines, and one under the current varietal registration scheme. The current varietal registration scheme requires data from three years

of field tests in different regions of the country, which demonstrate the agronomic advantages of the new variety.

None of the scientists the team spoke with expressed concerns about the risks of field testing or consuming biotechnological products. Nevertheless, the scientists involved in field testing are using a step-wise approach in their experimentation, evaluating each set of results from field experiments before enlarging acreage in subsequent years. Thus, the standard "good agronomic practices" are functioning as an informal set of biosafety principles.

Intellectual Property Rights

The Ministry of Agriculture also acknowledged that China's insufficient provisions for the protection of intellectual property. The Ministry said that China is considering new plant varietal protection measures. China has internal patents, and has just joined the World Intellectual Property Organization.

The team gained the impression that China clearly wishes to be an active participant in the projected international biotechnology market place, and recognizes that protection of intellectual property is a fundamental prerequisite for such participation. This point was emphasized by the Director of the impressive Food Quality Inspection Center at the Hubei Academy of Agricultural Sciences in Wuhan. He stressed that the Chinese Government is eager to interact with technical experts in the United States who are responsible for setting standards for agricultural products, so that China can become more integrated into the world trading system.

The Status of Field Testing

China had conducted many small-scale field tests of genetically modified plants and associated microorganisms. Among the species reported or observed by the team in the field were rice, maize, wheat, cotton, barley, soybeans, peanuts, alfalfa, grapes, tomatoes, tobacco, rhizobia and an extensive array of vegetable crops. Small-scale field tests are normally performed in the experimental fields of the research institution and are supervised by the principal investigator.

If initial tests are successful, researchers working through provincial ministries of agriculture, rent land from farmers to conduct larger field tests and subsequent full-scale performance trials. Two genetically engineered organisms were reported to be at the stage of full-scale performance trails -- virus resistance tobacco and an improved nitrogen-fixing rhizobium.

The virus resistant tobacco work is being done at the CAS Microbiology Institute of the in Beijing. This laboratory has traditionally worked on plant viruses and has targeted tobacco. Transgenic plant work began 8 years ago, and in 1986 tobacco with coat protein-mediated resistance to tobacco mosaic virus (TMV) was produced. This virus and cucumber mosaic virus (CMV) are extremely important in China.

Field tests were started in 1989, with winter tests on Hainan Island. Flue-cured tobacco (U.S. varieties NC89 and NC364) are currently in the fourth generation and are being tested for chemical constituents and smoking quality. The TMV resistance is excellent, but CMV coat protein is expressed at only one-hundredth of the level of TMV. Thus, the goal is to raise the level of expression and resistance to CMV. There has been no detected change in phenotype and chemical composition. Some lines are now to 6 generations, and in 1992 were planted to about 10,000 hectares. The size of the field tests in 1993 is larger, but no exact data were available on

area or yield. Generally, infection was lower in 1993 than in 1992, so it was not as good a test year. The tobacco produced on the 10,000 hectares was harvested and processed for cigarettes.

Major funding for the tobacco research was provided by the 863 program under the 1985-1990 Five Year Plan. The 863 is currently only providing one third of the funding necessary to continue the project, so funding from other sources is also being used. The China Tobacco Company, a state monopoly, is conducting economic analyses of the results of the most recent field tests. Given the advanced state of testing, it seems likely that an application will be made to commercialize this product in China during the coming year.

The other product which has been tested in very large plots is a genetically modified rhizobium. The project, which is being carried out by the Agro-Microbiology Department at Huazhong Agricultural University in Wuhan, is focussed on creating highly efficient strains of soybean and groundnut (peanut) rhizobia. In 1990 - 1992, a genetically engineered strain (HN32) of soybean rhizobia was tested in Hunan province on 10,000, 30,000 and 32,000 hectares respectively. Data from early results indicate that yields with HN32 were 2945.3 kilograms (kg) per hectare (ha), compared to 2731.5 kg/ha for a non-engineered strain of *Rhizobium japonicum* (2210), and 2522.2 kg/ha for the natural population (control). Tests are currently underway to examine the compatibility of HN32 with insecticides used on soybeans, and to assess the effectiveness of HN32 on peanuts in pot studies.

Although official permission has not yet been sought for commercialization, the recombinant rhizobia, HN32, is now being produced in a factory for use in four locations in southwestern and northeastern China. In terms of processing and consumption, soybeans harvested from experimental plots are apparently not differentiated from other soybeans.

Economic Implications

China has the potential to effectively apply biotechnology to boost the quantity and quality of it agricultural production. All indications are that the biosafety guidelines under development will be quite liberal, and will not "over regulate" applications of biotechnology to agriculture. There is not likely to be a problem with public acceptance of biotechnology products in China..

If China is successful in applying the techniques of modern biology to their agricultural sector, this will undoubtedly have an impact on U.S. - China agricultural trade. China's remarkable economic growth is already being reflected in world agricultural markets. In 1992, China's agricultural exports grew by 45 percent (increased exports were valued at $15.26 billion), and imports grew by 43 percent ($8.65 billion).[2]

Major expansion of Chinese exports came from cereals, tobacco and cigarettes sales. As discussed above, transgenic tobacco is nearing commercialization in China and should enhance their competitiveness in world markets if it is combined with improved quality. China's primary increases in cereal exports were maize and rice to Asian markets. Both these commodities are primary targets of China's biotechnology research program.

China's main imports from the United States are wheat and cotton. Wheat trade is unlikely to be affected by this new technology for the foreseeable future. Cotton, on the other hand, is a target species for biotechnology

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[2]International Agriculture and Trade Reports, China, USDA/Economic Research Service, July 1993, p.6.

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applications in China in the current Five Year Plan. China's cotton production in 1993 was curtailed, in part, by a major cotton bollworm infestation. Biotechnology potentially offers a partial solution to this problem, Other key areas which are likely to impacted biotechnology in the future include the rapidly growing livestock and feed sectors and the edible oils markets.

From the economic perspective, biotechnology offers many advantages to China's agricultural sector. China's input delivery system is changing form one directed by central planners to one driven by market forces. Rising fertilizer prices and transportation bottlenecks have curtailed the purchases of fertilizers and some other inputs.[3]

The advantage offered by biotechnology is that improvements can be built-in to new varieties which can produced in China in regional centers, decreasing the need for importing and trans-shipping agricultural chemicals. It is likely that research institutes and universities throughout the country will produce and distribute biotechnology products directly to farmers, since they are already doing this for non-engineered biocontrol agents and other products.

Conclusions and Recommendations

The team's primary recommendation is that the United States should increase its interactions with China in agricultural biotechnology. This should encompass: (1) increased consultation on biosafety and intellectual property rights issues, (2) increased collaboration in selected scientific and technical areas, (3) closer consultations on biotechnology policy in order to enhance the broader relationship between U.S. and Chinese agricultural communities, and (4) exploring ways to assist the U.S. agricultural biotechnology industry in gaining knowledge about opportunities for joint ventures in China.

Specific Recommendations for Follow-up include:

a. Generate proposals for follow-up team visits to China under the exchange program to explore a number of areas of potential mutual benefit in greater depth. Specific topics for follow-up team visits would be:

(1) a rice apomixis survey (proposal currently being developed by Dr. Rutger);
(2) aquaculture and biotechnology (approved for the 1994 exchange program);
(3) transgenic animal survey (1995);
(4) a follow up plant biotechnology survey visit (1995);
(5) a biocontrol survey; and
(6) a enhanced nitrogen fixation team.

b. From the findings of this and subsequent team visits explore the possibility of collaborative research such as:

(1) rice apomixis investigations;
(2) soybean biotechnology, germplasm, and virus resistance;

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[3] Ibid, p. 8.

(3) enhanced biocontrol organisms (Bt, streptomyces, etc); etc.

c. Explore linkages in information systems. Chinese biotechnology laboratories could benefit greatly from computerized "Silver Platter" literature search capabilities and access to other scientific information systems in the United States. Such facilities and information are available from the National Agricultural Library and university libraries. Likewise, the United States could benefit greatly from access to information about Chinese research projects. Collaboration in this area would entail technical assistance to establish a current biotechnology information database in China.

d. To build mutual understanding, exchange information on biosafety, intellectual property rights and other biotechnology policy issues with key players in China. Actively solicit Chinese participation in the key international meetings such as the Third International Symposium on Biosafety Results of Field Tests of Genetically Modified Plants and Microorganisms, which will be held in November 1994 in Monterey, California.

e. Explore the possibility with the Agricultural Marketing Service of interactions with the Food Quality Inspection Center in Wuhan.

f. Circulate this report, the reports of subsequent biotechnology activities and provide oral briefings to the U.S. scientific community, including to U.S. agricultural biotechnology companies.

g. Working with the USDA Office of Public Affairs, pursue the possibility of a joint U.S. -China publication on biotechnology research highlighting specific Chinese research activities which are unique and not well known in the United States.

Appendix A

ITINERARY

September 17, 1993

Ministry of Agriculture, Beijing
Biotechnology Research Center, Chinese Academy of Agricultural Sciences, Beijing
Germplasm Institute, Chinese Academy of Agricultural Sciences, Beijing

September 18, 1993

Institute of Microbiology, Chinese Academy of Sciences, Beijing
College of Biological Sciences, Beijing Agricultural University

September 20, 1993

China National Center for Biotechnology Development, Beijing

September 22, 1993

Nanjing Agricultural University

September 23, 1993

Jiangsu Academy of Agricultural Sciences, Nanjing

September 24, 1993

Jiangsu Agricultural University, Yangzhou

September 25, 1993

The Forestry Institute of Jiangsu Province, Nanjing

September 27, 1993

Huazhong Agricultural University, Wuhan

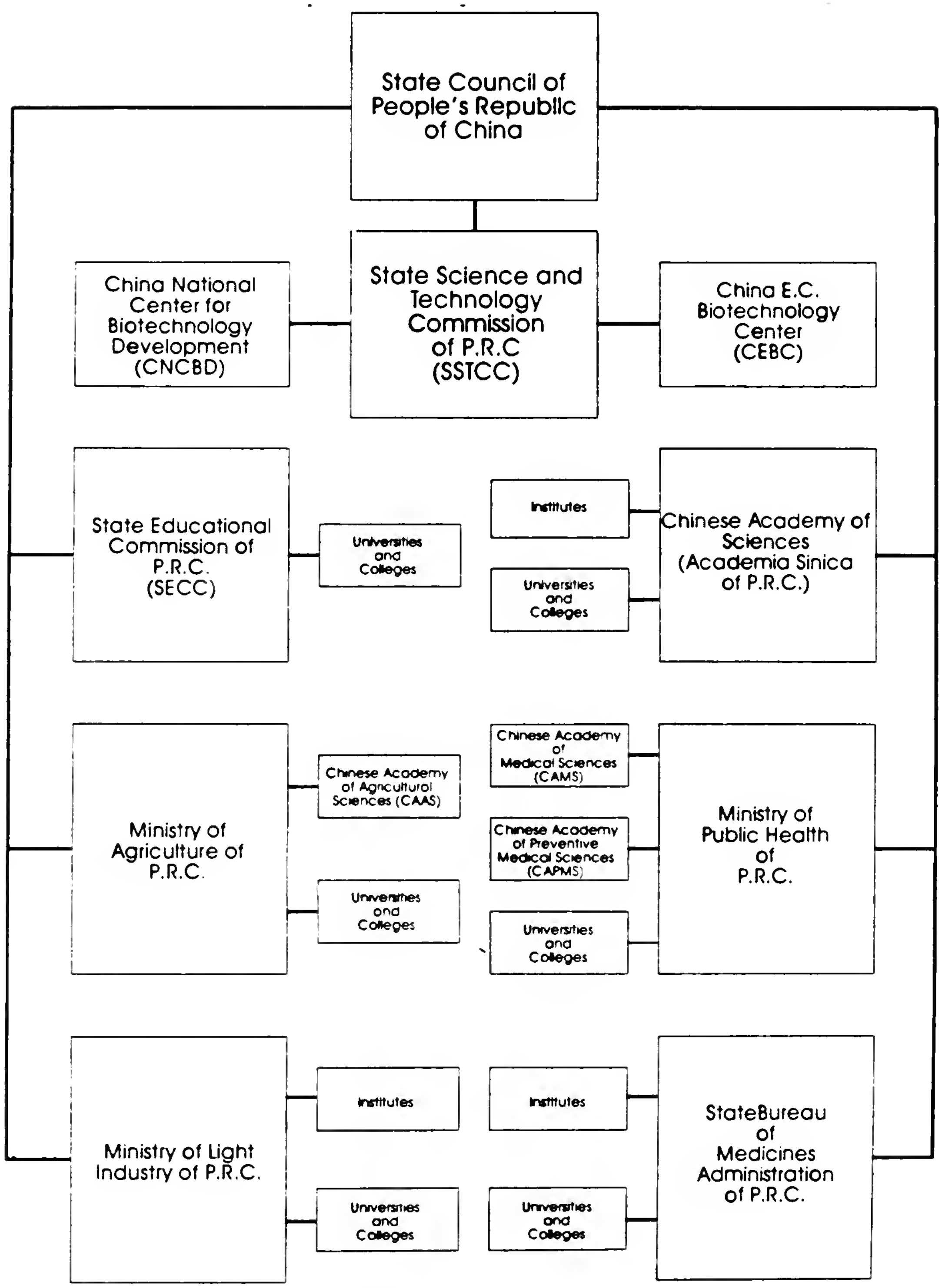
September 28, 1993

Hubei Academy of Agricultural Sciences, Wuhan

September 29, 1993

South China Agricultural University, Guangzhou
South China Institute of Botany, Chinese Academy of Sciences

ORGANIZATION CHART OF BIOTECHNOLOGY ADMINISTRATION AND RESEARCH[4]



[4] China-EC Biotechnology Newsletter, September 1992, P. 29.

Detailed Reports of Administrative and Laboratory Visits

9/17/93 <u>Ministry of Agriculture, Beijing</u>

Zhang Junlong, Agronomist/Deputy Director, Department of Academic Activity and Deputy Secretary General, Chinese Society Of Agrobiotechnology, gave an excellent briefing of agricultural biotechnology in China. Also present was **Wang Jian**, Deputy Division Director, Department of International Cooperation.

Ministry of Agriculture, SSTC, CAS and Ministry of Public Health have joint responsibility for biotechnology in China. Programs began in 1986. Three funding sources are the Ministry, Commission of Planning and the High Tech Program. Ministry is the smallest (12-15 %). Six national laboratories are funded by State Commission of Planning. The plant biotechnology lab at Huazhong Agricultural University is one.

Ministry has an advisory committee of experts from around the country. They would like to have foreigners serve, but there are "technical problems" in implementing this idea. Proposals are peer-reviewed by an advisory committee.

Guidelines for assuring safety of field releases are under development. After general guidelines are accepted, then specific guidelines for sectoral applications will be drawn up. The Ministry will have responsibility for plants and microbes related to agriculture. The People's Congress must approve Guidelines.

Five Year Plan (1991-95) sets out techniques to be employed including genetic engineering of plants and animals, micropropagation, embryo transfer, *in vitro* fertilization, recombinant vaccine development, and monoclonal antibody development. These techniques are to be applied to increase yields and to improve the quality of agricultural products.

Department of Education operates 16 universities, 8 are key. CAS operates national labs; some at universities. Thirteen provincial academies of agriculture are also doing research.

Officially, products of biotechnology have not yet been approved for general use by farmers. Some may be in use at small-scale. No applications for commercialization of biotechnology products have been received yet by the Ministry. They will require two clearances -- one for biosafety and one normal. China is considering new variety protection for intellectual property.

The Torch program is aimed at technology transfer. Monsanto has visited China and is interested in biotechnology development. Bioprocessing is weak in China. They are still looking for food applications. There is some industrial production of biogas. Plant varieties are not patentable in China, but processes involving microbes are patentable. Nationally, germplasm is maintained by CAAS.

After the briefing, the team was hosted at a banquet in the Ministry by **Liu Congmeng**, Deputy Director-General, Department of International Cooperation. He expressed interest in joint publication on biotechnology.

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9/17/93 <u>Chinese Academy of Agricultural Sciences, Biotechnology Research Center, Beijing</u>

The Biotechnology Research Center is one of the 37 institutes within the CAAS. It was established in 1986 by drawing together, in one location, about 80 staff -- scientists, technical persons, and support staff. There are 4 sections: (1) a research laboratory for molecular biology; (2) a research laboratory for cell biology; (3) a technology development group; and (4) a workshop to produce monoclonal antibodies. The overview of the center was given by **Yu Jiu-Jun**. Individual scientists presented their research areas and progress to date. The research appeared to be state-of-the art with regard to the areas chosen and approaches taken. In many cases, it had direct application to agricultural productivity. The facilities of the first two sections were shown to the team. This was the team's first view of laboratories. They were quite proud of a "gene gun" that was constructed in China from inexpensive components, and transgenic plants (tobacco, rice) growing in Erlenmeyer flasks.

The labs were generally spacious, with only 1-2 equipment items per room of 100 ft^2. The basic items of equipment to conduct cellular and molecular biology research appeared to be present. However, it was not being utilized during the visit.

The Cell Biology group, as described by **Jia Shi-Rong**, is quite active. They have identified a small peptide gene of 35-37 amino acids, which has anti-bacterial properties to *Pseudomonas*, *Erwinia*, and *Agrobacterium* species. They currently have produced about 300 potato lines carrying this gene and are progressing to develop these lines further. They also have programs to develop transformation systems for several agronomic crops, with current focus on potato and tobacco. Since 1987, seven crops have been transformed successfully, apparently in this laboratory. They described an interesting basic research project to transfer the elusive "phytochrome" gene from rice and tobacco into the ornamental *Kalanchoe*. Work was progressing to improve the transformation efficiency from the current 2-6% in cucurbits, and to insert part the polymerase gene of zucchini yellow mosaic virus to induce resistance. A new method of transformation utilizing ultrasonication was showing initial promise.

In the Molecular Biology group, **Meinan Yu** described work, supported by the 863 plan, to increase protein productivity of plants by increasing the content of sulfur (S)- containing amino acids (prolamine) in animal feeds to ultimately improve wool quality. She has succeeded in increasing S by 10% in *Lotus* (birdsfoot trefoil), as detected by Southern and Western blots. **Liu De Hu** emphasized work with plant viruses, and is seeking a new approach to introducing resistance to potato virus Y into tobacco and potato by inserting a monoclonal antibody gene into the plant genome.

Fan Yun-Liu and her post doc, **Qi Chen**, were generating plants resistant to Lepidopteran pests and were looking toward a long-term management strategy utilizing different Bt genes (had contact with Monsanto) and proteinase inhibitors (made themselves with outside advice), as well as various promoters and other factors. They sought cooperators for tissue-specific promoters. The group has recently published an article in the British journal <u>TRANSGENIC RESEARCH</u>, and seemed to be enthusiastic and well-informed. A basic project involving the regulation of gene expression in *Brassica* and cytoplasmic male sterility (CMS) has begun. They are seeking a stable CMS system, embryonically expressed, which cannot be overcome by high temperatures, as well as to elucidate the role of the ATP-6 mitochondria gene in CMS.

<u>National Gene Bank</u> - During the afternoon visit to the Chinese Academy of Agricultural Sciences (CAAS) in Beijing, the team briefly visited the National Gene Bank. The Bank was completed in 1988, and now houses

227,839 seed collections. It is the counterpart to the National Seed Storage Laboratory in the U.S. Of the total Bank entries, 50,790 are rice, 34,883 are wheat, 20,645 are millet, and 18,300 are grain soybeans. There also was a small collection of vegetable soybeans, and 3,626 entries labelled "Oryza", which may have been wild species of rice. Long-term storage was maintained at -18°C; an intermediary storage was at 5°C.

The data systems operator showed the team a state-of-the-art germplasm information system; which, for example, pictorially displayed the number of accessions from each province and district of China. In response to a question of how many rice entries were keng (japonica, or temperate zone rice), the operator ran a tally of 15,138; the remaining entries presumably were hsien (indica, or tropical rice). The keng (japonica) rices are the predominant type grown in the U.S.

9/18/93 Chinese Academy of Science, Microbiology Institute, Beijing

This laboratory has traditionally worked on plant viruses and has targeted tobacco. The overall program was presented by **Rong-Xiang Fang**, an articulate scientist who retains a close association with Rockefeller University in New York. There are 20 scientists in his group (no technicians since the cultural revolution), thus, students do most of the work. Dr. Fang heads half of the lab; **Tien Po**, who was not present, heads the other.

Transgenic plants work was begun 8 years ago, and in 1986 tobacco with coat protein-mediated resistance to tobacco mosaic virus (TMV) was produced. This virus and cucumber mosaic virus (CMV) are extremely important in China. Field tests were started in 1989, with winter tests on Hainan Island. Flue-cured tobacco of the U. S. varieties NC89 and NC364 are currently in the fourth generation and are being tested for chemical constituents and smoking quality. The TMV resistance is excellent, but CMV coat protein is expressed at only one-hundredth of the level of TMV. Thus, their goal is to raise the level of expression and resistance to CMV. The have detected no change in phenotype and chemical composition. Some lines are now to 6 generations, and in 1992 were planted to about 10,000 hectares. They are conducting economic analyses. For 1993, they were not sure of the total area or on the yield. Generally, infection was lower in 1993 than in 1992, so it was not as good a test year. In response to the question of the fate of the tobacco produced on the 10,000 hectares, they said some of it could have gone into production of commercially available cigarettes. In central China, TMV/CMV resistant plants also show some resistance to PVY. In a northern area where only TMV is prevalent, the plants with the TMV construct alone are performing quite well.

Work was also described with the goal of producing virus-resistant vegetables. Tomato resistant to TMV/CMV, peppers to CMV, potato to PVY and other viruses have been produced. They were the first lab to publish the sequence of turnip mosaic virus (TuMV), an important virus in *Brassica*, and are supplying constructs to a lab in the Institute of Genetics (which the team did not visit) for insertion into *B. napus*. They are also cooperating with a lab in northwest China for producing and testing transgenic peppers.

Pioneering work was being done on rice yellow stunt virus (RYSV), a plant Rhabdovirus that has not been previously purified and characterized. A project begun in 1988 with Rockefeller support has purified the virus (1 year), isolated the 12 KB mRNA (another year), and now have a cDNA library of the virus. They are producing monoclonal antibodies to non-capsid proteins and are cloning and sequencing the N gene (the gene in tobacco conferring resistance to TMV). They have targeted the N gene to introduce into rice, in

cooperation with the Institute for Genetics and using the gene gun, to induce protection. This gene was chosen because it was found effective for protecting plants to tomato spotted wilt virus. They are the only lab in the world working on this virus, now that Japanese workers have quit because they were unsuccessful in isolating sufficient virus from plants.

There are additional groups working on insect resistance in tobacco and poplar mediated by Bt toxin genes. Other virus work includes a ssDNA virus in banana, and fan leaf and leafroll viruses of grapevines. Genes have been isolated and constructs are currently being made for insertion into plants by a lab in northeastern China.

Additional basic work is being done to examine defense genes in plants, primarily rice. They have isolated glycine-rich proteins and signal sequence, and found that the HS70 promoter is also induced by virus infection. These and chitinase and phenlammobialyase are induced by RYSV, even though no necrotic symptoms are induced.

New projects include a search for antibacterial and antifungal genes, and examination of promoters and constructs to improve their results. One focus of this work is expressing two transgenes at the same time. This was an extremely enthusiastic group. The labs were well-equipped with several work stations occupied by graduate students.

The efforts of the Enzymology Department were described. These consisted of the preparation of enzymes for use in Industrial Microbiology, including pectinase in fruit juice and wine, asparaginase for use as an anti-tumor agent, aromatic amino acid syntheses, and improving the enzyme chymosin by genetic engineering.

The second part of the Microbiology Institute, Tien Po's group, was described by **Zhang Yong Quing**, a scientist trained in animal virology but who recently transferred to this group. The major projects included (1) CMV and satellite RNA as a biological control agent; (2) novel engineered resistance using tomato and tobacco, rice, and potato; (3) genetic engineering for male sterility and fertility restoration in tobacco, oilseed rape, cotton and maize; (4) fruit ripening controlled by transgenes of the ACC synthase pathway in tomato, tobacco, honeymelon, and grape; and (5) antibody engineering, in which plants act as bioreactors to produce human immunogens, such as hepatitis A antigen (just starting). Several posters describing their results were on display in the laboratories. A painting and renovation project was underway, which made it difficult to observe the facilities.

9/18/93 <u>Beijing Agricultural University, National Biotechnology Laboratory, Beijing</u>

This is one of six national laboratories, and includes (1) transgenic animals; (2) plant tissue culture, wide hybridization with medicinal and industrial plants, studies to improve grain quality of maize; (3) molecular virology; and (4) microbiology - nitrogen fixation, fermentation, edible fungi. There are also other laboratories and scientists throughout the university. Up to 10 visiting scholars come each year and up to 140 projects are underway.

Basic research was being conducted on maize transformation via microinjection, particle gun bombardment, and ultrasonication. The second generation of corn plants were seen in the field that had been transformed by ultrasonication of callus. Other maize work was on the mechanism of heterosis, European corn borer resistance in collaboration with Ray Wu (Cornell) using his proteinase inhibitor gene construct, and improved

quality for higher lysine and S-containing amino acids. Virology research by **Dr. Yu** concentrated on fungal-transmitted viruses, particularly wheat yellow mosaic virus. They had made transgenic tobacco, sugar beet and carnation, and were looking at genes other than the coat protein gene, to induce resistance.

The labs were extensive, well equipped. They were located on the sixth and eight floors of the main building. Instrumentation rooms were impressive, with Beckman J21, L8-80, L7 centrifuges, various power supplies (including Chinese manufacturers, as well as LKB, Biorad, etc.) They originally had the equivalent of $1 million U.S. to set it up. Computers were in a separate room. The virology lab, transgenic animal lab and the nitrogen fixation lab were among the best the team saw, and were occupied by active researchers. The focus in the animal area was utilizing pigs, rabbits and others as bioreactors to produce insulin, growth hormone and other compounds. They were pushing China as a collaborator in such research because research costs are quite low to house the animals.

9/20/93 China National Center for Biotechnology Development, Beijing

The team was received and briefed by **Ding Yong**, Deputy Director, a key player in Chinese policy development for biotechnology. Also present were **Xu Xinlai**, Associate Professor, and **Li Qing**, Assistant Researcher. The meeting took place in the lovely conference room of the China - EC Biotechnology Center, which has its offices in the same building. This is also the China branch of the UNIDO organization based in Italy and India.

The briefing covered the biotechnology research structure and policy issues in China.
The CNCBD is administered by the SSTC. Biotechnology is an important issue at the national level. Director Ding noted they have three priorities: (1) agricultural biotechnology; (2) medicinal biotechnology, and (3) protein engineering.

They undergo a TQM-like process to select "863" grants. They are using the FDA and NIH biosafety models.

Within China, the rice genome project is funded by SSTC. Work is being carried out at a number of centers including Shanghai National Academy of Sciences, Beijing University/Institute of Genetics, Huazhong Agricultural University, the China National Rice Research Institute in Hangzhou (CNNRI) and Fudan University.

9/22/93 Nanjing Agricultural University, Nanjing

The team was welcomed by **Yan Zhiming**, Deputy Director of the International Exchange Office. He was joined by scientists in Genetics and Plant Breeding, the Soybean Research Institute, and the Microbiology Research Laboratory. A video (made in 1988) of about 20 minutes long described the University and its many programs. There are currently about 6000 students and over 900 teaching and research staff of which about 330 are Professors, Associates or Senior Researchers. This institution has a long history, but moved to its current site only in 1958. They have long held cooperating agreements with major agricultural institutions worldwide.

Several projects were described that dealt with plant genetics and breeding. It was clear that researchers in this group are starting to combine tools of cellular and molecular biology with those of classical genetics. About 20 people were involved with rice, although only one of them was present. **S. H. Yang** described his

work with rice leaf blast that had begun in 1987. Mutants generated of the causal fungus, *Piruculaia*, by transposon tagging, and Hrp and Sp genes induced in the plant, are being investigated. The goal is to map these resistance genes by RFLP or RAPD analyses, and to characterize the product of the gene by isolating the plasma membrane in which it is localized. The main project in the rice lab is to generate high protein lines through genetic engineering. To facilitate this, he is attempting to increase the regeneration frequency in indica lines by selection following somoclonal variation.

A project involving wide crosses for wheat improvement is being elegantly conducted by **Chen Peidu**. Traits sought include Hessian fly and powdery mildew resistance, obtained from *Haynaldia villosa*, and resistance from *Agropyron*. They obtained hybrids through techniques involving protoplast fusion and embryo rescue. A DNA probe specific for *H. villosa* was generated and used to follow the insertion vial RFLP analysis of these genes into wheat, in work done by a Ph. D. student there. A well-equipped and active cytogenetics research section had followed chromosome transfer and maintenance by the wheat lines. The goal, to locate and clone resistance genes, appears to be attainable by this large, well-equipped set of laboratories.

The soybean breeding program, housed in the Soybean Research Institute, was of particular interest to Dr. Tolin. Professor **Gai Junyi**, President of NAU and Deputy Director of the Institute, had visited the soybean research team at VPI&SU in 1992. However, he regretfully was on travel and unable to meet with the team. In addition, a scientist from the SRI, **MA Guorong**, is currently a Ph.D. student at VPI&SU with Dr. Tolin and her colleagues. The main project of **Hu Yun Zhu**, who was the only researcher available to meet with us because of a conflicting conference, was to examine the genetics of resistance to soybean mosaic virus, and important seed-borne virus. Focus was on virus strains, resistance sources, and genetics. Their results have shown resistance to 4 strains are on the same chromosome, but they do not think this is the same gene as has been found by U. S. researchers. The soybean group does not appear, as yet, to be using molecular approaches to this project. They are, however, examining male sterility. The team was shown a soybean germplasm collection of over 4000 entries kept in sealed glass jars at room temperature, and informed that the main supply was kept at low temperature. Cursory examination of many of the jars revealed a high frequency of seed-coat mottling often associated with seed-borne SMV infection.

The tobacco breeding program, described by **Huqu Zhai**, is also using wide crosses to transfer useful genes from Chinese herbs into tobacco. The project is now using *Gymnostemma pentophyllum*, which contains an as yet unidentified compound used for heart ailments and antibody stimulation. An HPLC had just been installed the previous week to aid in the purification of this compound. The work was presented at the International Genetics Congress in Scotland and received wide attention.

Guo-Ping Yang, soil microbiologist, described microbiology research. The Microbiology Dept. has a staff of 22 with two Ph.D advisors. The number of Ph.D. advisors in China is strictly controlled by the Central Government. (At a later visit the team learned that this system is currently under review.) Soil microbiology research deals mainly with N-fixation, anaerobic processes, degradation of agricultural chemicals, mushroom research, mycoplasmas and viruses, and industrial microbiology. Dr. Yang's personal research involves the physiology and genetics of N-fixation by nodulation organisms and signal exchange between microbes and plants.

Dr. Yang noted that although N-fixation work is being done at a number of sites, the most active and productive is at the Chinese Academy of Sciences in Shanghai in the Biochemistry and Plant Physiology Departments.

Dr. Yang described some of the work of **Ching-Seng Fan** (who was visiting the United States) on Effective Microorganisms (EM). Dr. Fan believes that the EM product he is testing has too diverse a collection of microorganisms in it. The product is giving variable reactions in different soil types. Dr. Fan is getting more consistent results with his own cultures.

Dr. Yang also showed Dr. Kaufman samples of a packaged product manufactured by the Department for vegetable seed treatment. The product was labeled BC2 and is used to control plant diseases, promote plant growth and increase yields.

In general, the labs were neat and orderly, and a second building designated for Isotopes (but including several other areas of research) was particularly well-kept. Equipment included: centrifuges (Beckman J21, ultra), microfuges, DNA synthesizer, Beckman DU-7 spectrophotometer, new protein purification HPLC, many micrcscopes with some having camera backs, power supplies and electrophoresis apparati. Autoclaves were small "pressure cookers". The cytogenetics lab was quite crowded and active, with several women who continued looking through their scopes while the team was there for an extensive discussion.

9/23/93 Jiangsu Academy of Agricultural Sciences, Nanjing

Fang Xin-Yi, Deputy Chief of International Relations, explained that JAAS was founded in 1932, and has taken the present name since 1977. JAAS consists of 12 institutes plus an English Training Center at this location, and has over 1000 staff in Nanjing. In addition, 9 regional agricultural institutes and 6 vegetable research institutes are administered jointly by JAAS and localities within Jiangsu province. The Institute of Agrobiological Genetics and Physiology was described to the team by **Xue Qi Han**. The emphasis was on basic research and included molecular genetics, cytogenetics, physiology, developmental biology, and biochemistry. Biotechnology is a recent emphasis, with cellular and molecular methods being applied to practical plant breeding problems. Applied research such as variety development is done by other institutes. Achievements described by Dr. Xue and by **Zhang Deyu** include RFLP and RAPD analysis of disease resistance genes in wheat and rice, and identification by RFLP and tagging of a gene in rice for wide incompatibility, cooperatively with Cornell (cloning by letter). They are working toward *Agrobacterium* transformation systems for sweet potato, cotton, *Brassica repens* and other vegetables and transformation by electroporation for rice and wheat. Interest in collaborating with scientists in the U.S. and other countries was expressed, particularly for genes for resistance to fungal diseases, virus coat protein genes. With sweet potato feathery mottle virus, they have synthesized cDNA but have no funds to proceed toward sequencing the viral genome and transforming sweet potato.

Dr. Zhang also described his earlier work with soybean glyceollin genes done with Japanese scientists, and current work using the Cambridge (U.K.) system for In situ hybridization using specific biotin-labeled probes, either total DNA or specific fragments, he has generated to tag chromosome segments exchanged wide crosses of wheat. In an RFLP analysis of wheat lines isogenic for powdery mildew resistance, he has obtained 3 polymorphic markers and is mapping the R gene.

Ni Wanchao of the Institute of Industrial Crops described his work in cotton biotechnology. Wide crosses have been attempted with cotton and 38 wild species, seeking fungal disease (*Fusarium oxysporum*) and pest resistance and improved fiber quality. They accomplished transformation 4 years ago by pollen tube transfer, first using total DNA from wild species and later genes cloned by Taiwan University scientists. Plants transgenic for BT toxin or proteinase inhibitor (PI) have been obtained. The 5th generation of plants is now

being field tested and showing good insect resistance. They are now using modified Bt's and considering combining it was Pi, to increase the durability of the resistance. They are collaborating with Shanghai University and Beijing Agricultural Academy of Science Biotechnology Center, and said Monsanto had been in contact with them about the work. Plans are to extend the work to include herbicide resistance. Research with soybean and oilseed is also conducted in this institute. Soybean transformation had been attempted, but proved more difficult. They mainly supply soybean germplasm to other institutes.

During the tour of labs and the experimental farm, it was noted that some work was done on rice in the Institute of Food Crops, and that hybrid rice was being developed by the 3-line approach. It was also stated that people in cities prefer japonicas because of poor quality of indicas, especially hybrids. There was extensive tissue culture work, including micropropagation of lilies and squash. Extensive ponds on the site were used to grow *Spirilla*, which is dried, pressed into cakes and used either for direct consumption or for carbohydrate extraction. It is also used for cosmetics.

The scientists at JAAS seemed to have good cooperation with the neighboring Nanjing Agricultural University and with the provincial Jiangau Agricultural College in Yangzhou (to be visited next) and students often worked in their laboratories. In addition, scientists at the institute were permitted to earn extra income through consultation with private industry, including multi-national companies. Some of this, perhaps, was connected to the role of JAAS as a testing group for chemical registrations. Overall, the scientists at this location were an active group, widely cooperating with scientists within and outside of China, to apply cellular and molecular tools to practical plant breeding problems.

9/24/93 <u>Jiangsu Agricultural University, Yangzhou City</u>

This is a good location, enthusiastic, but no transgenic plant work was being done there as yet. **Ming-Hong Gu**, Vice President of JAU and Director of the Biotechnical Institute, met us at the door and presented Dr. Rutger with a picture of the two of them taken at Beaumont, TX, in 1983. He studied at Kansas State with Dr. George Liang.

Prof. Gu briefed the team: JAU has 3,000 students, 550 faculty, 6 departments, and 2 special divisions. There are more than 70 colleges and universities in Jiangsu Province, of which 5 are key universities. JAU is one of the 5. They have two key courses of study: plant breeding and genetics, and veterinary medicine. Besides teaching, they have 198 research projects: 10 national level projects, 5 with "863" on high tech, 20 with National Foundation of Natural Sciences, 7 Ministry of Agriculture of China. Total research funding is 3.2M yuan/year (ca $0.5M), excluding salaries.

They try to send 5 to 10 young faculty members abroad per year. We met Associate Professor **Pan Xuebiao**, who wants to go to the U.S. for a year to work on wide compatibility genes in rice.

They have made transgenic rabbits and goats (HGH), but not crops, although they are doing extensive tissue culture. The team heard about two improved peach varieties from tissue culture, also meristem culture of Lotus to produce virus-free stocks. The white-flowered China lotus is used widely, consumed by humans as a high starch, vitamin and protein food. Over 150 varieties are distributed from JAU, and exported to Japan, Hong Kong, and Southeast Asia.

Peng Yongxin, who recently spent a year at LSU, described the new corn male sterile cytoplasm - Y type - that he discovered in 1978. This cytoplasm is different from the corn blight susceptible T cytoplasm, also different from C and E types, by mitochondrial DNA restriction maps. The Y type is highly resistant to *Helminthosporium*, the corn blight fungus. He developed a single cross hybrid, Su Yi 6, which is now grown on 300,000 hectares. The Y type has been described in the Maize Genetics Newsletter, but China has not made it available to western seed companies. Seed production is handled by the Great Wall Seed Company in Hubei Province, which is one of the largest seed companies in China.

Prof. Gu described three 863 projects on rice: (1.) use of wide compatibility genes for making fertile indica x japonica crosses. H.Ikihashi in Japan originally described one wide compatibility gene; they have 2 or 3 more. They are trying to pyramid these genes. (2.) converting hybrid rice from the 3-line to the 2-line system. Prof. Gu gave Dr. Rutger a recent book of papers on this; and (3.) RFLP mapping of key genes related to fertility, sterility, and restorer factors.

In the afternoon, the team toured labs and field plots, where the team saw the Su Yi 6 corn, and a new scented, glutinous rice, Guang Ling. This was the first time that Dr. Rutger had seen scented and glutinous combined. It was a japonica short grain, semidwarf variety.

9/25/93 <u>Forestry Institute of Jiangsu Province, near Nanjing</u>

This institute is a beautiful remote location dedicated primarily to tissue culture and micropropagation of forest trees (willow, poplar, paulonia); horticultural flowers (roses, carnation, African chrysanthemum, etc); medicinal herbs (pearl orchids); and vegetables and fruits (strawberry, kiwi). It is one of the largest in the country, finished in 1990, it has 22 culture rooms, each 388 square meters and a 1000 square meter greenhouse. It is the site of the Lu Bao Corporation, which generates income by providing services and plant propagative material on a large scale. It serves as the gene bank for willow and kiwi. The fruit tree gene bank is in Henan Province.

Only a small portion of the labs seem to be used at present. They are not presently testing for virus or other pathogens, but they are looking for cooperation with quarantine labs. Selection for improved varieties is done mainly by traditional methods in Beijing, and this lab just propagates for commercial use. There are 89 total staff. One staff member who studied in Italy is considering genetic engineering of poplar, but does not yet have funds to do it.

Ni Jing De, Associate Manager of the Jiangsu Lu Bao Plant Micropropagation Corporation, explained that the JPFI began in 1986 and the Corporation was set up in late 1992. It is designed to produce 2 to 3 million forest tree seedlings/year from tissue culture.

Zhu Lu-Ming and **Liu Gonglin** explained they were micropropagating a medicinal herb plant (pearl orchid, *Anoectechins formosamus*), for export to Taiwan as a shoot in a flask, and kiwi fruit trees. They have clonal germplasm plantings of willow (900 entries), poplar, and Chinese fir. Cut flowers are a new market for China, and roses were produced in their extensive and impressive greenhouses in a soilless propagation system with micro-chip controlled nutrient solution drip irrigation. An export market is apparently developing. This lab may be a good prospective partner for a joint venture with an American partner.

Huazhong Agricultural University, Wuhan

Wang Yunhua, Vice President, and a group of about 20 scientists welcomed us. Dr Tolin met colleagues, **Zhang Qifa** of HAU, and M. A. Saghai Maroof, visiting from VPI&SU and a collaborator on the rice genome mapping project. HAU has nearly 6,000 students and covers 500 hectares. There are 2,399 staff, of whom 812 are teachers. HAU is among the first group of universities in China which is eligible to confer Ph.D. and M.A. degrees. It has trained more than 18, 000 graduates and special trainees. The university has 14 departments, 9 research institutes, 2 research centers, and 8 research sections.

Biotechnology at HAU began in 1980's for the purpose of training teachers and researchers, and building up laboratories. The former president at HAU was the Director of a biotechnology committee which sent scientists abroad to study, many of whom had returned and were present to visit with the team. The goal was to make an effort to advance science and narrow the gap between HAU and the rest of the world. A Biotechnology Center was established in 1992, with a Crop Genetic Improvement Lab an open laboratory of the university, as well as a satellite lab for the Rice Genome Mapping Project. Two other labs, Microbiology and Pig Genetic Improvement, were set up under the Ministry of Agriculture as key laboratories to all of China and to the outside. Research covers the areas of (1) plant molecular biology, (2) plant protoplasts, (3) microbiology, and (4) animal molecular biology. Support is from the National High Tech program - the "climbing" project; The Natural Science Foundation; and international cooperation of Rockefeller, EC, International Foundation for Science, World Health Organization, John Innes Institute, and Canada to name a few. The Plant Genetics and Improvement Lab has been ranked as one of the state key open labs. A new laboratory building is going to be constructed for biotechnology research. HAU has an active international program and has established collaborative relationships with Ohio State, University of California at Davis (UCD), Rutgers, and universities in New Zealand, Russia and Thailand.

In the morning, Prof. Zhang, Associate Director of the State Key Lab of Crop Genetic Improvement, and Director of the Biotechnology Center, showed the team their labs and had his colleagues make presentations: (1) **Jin De Min** discussed the Nongken 58S source of photosensitive genetic male sterility (PGMS) in rice. At Wuhan (30°27'N latitude), the critical day lengths ($\geq$ 50 lux) at 25°C are: $\geq$ 1400 hours - male sterile $\leq$ 1345 hours - male fertile; $>$1500 hr - male fertile. The sensitive stage for sterility/fertility is 25 to 15 days before heading. PGMS is controlled by 2 pairs of independent recessive genes (Nongken 58 had only 1 gene); (2) **Yao Jia Lin** discussed her work on the HDAR source of apomixis in rice, observing cytologically two types of egg sac formation. Apomixis frequency was 48% in twin seedlings, 16% in single seedlings; (3) **Yu Gongxin**, a lecturer in the Department of Agronomy who is going to Iowa State for his Ph.D., described his search for resistance to bacterial blight of rice. He tested 6,000 local rice from the area of origin of cultivated rice and found 345 resistant lines, only 80 of which were resistant to all 10 bacterial blight strains. At least 11 genes, some developmentally dependent, appear involved. Linkage analysis suggests Chromosome 7 for some genes. (4) **Shen Binzhang** is mapping PGMS with probes from Cornell. There was 12.88% seed set on the double recessive male sterile. The major gene was mapped to chromosome 7, the minor gene to chromosome 3. They now have markers on each side of each of the genes and proceeding to use YAC cloning to isolate the genes. (5) **Gao Youjun** is doing map-based Diallel analyses of heterosis in rice. Molecular markers may have predictive value for heterosis breeding, isozymes, RFLP probes, and microsatellites have been used to date. (6) **Liu Kede is** studying molecular characterization of rice germplasm. In samples from SW China rice diversity center, and of indica and japonica types, the diversity was greater with japonica but varied with the probes used and was not as much as had been expected. (7) **Dai Xiankai** (absent) is working on the Rice Genome Project. Professor Zhang expanded

further on the rice genome mapping project, and work being done by a student that was not present. They have set up a mapping population by making crosses between lines that are high polymorphic and those showing little segregation distortion. In China, there are 5 laboratories working on this project, 3 doing mapping and RFLP analysis, and 2 doing sequencing and cloning. They admist to being behind the Japanese, but they are proud to be doing something on their own and for their own rice populations. (8) **Li Jianseng** reported on Tibetan barley. They think barley is diphenetic in origin North Africa, and Tibet; (9) **Li Jiansheng** described a project on genetic diversity of maize is underway. Maize diversity in SW China source is greater than in U.S. maize! He is also looking to map CMS (cytoplasmic male sterility) in maize by RFLPs, and to map wheat specific defense response genes (chitinases and glucanases) induced by the tan spot fungus (*Pyrenophora tritici*).

After lunch the team visited the Agro-Microbiology Department under the able leadership of **Chen Haiku**. Dr. Chen is an extremely articulate and knowledgeable octogenarian, who speaks perfect Oxford English and is conversant in the latest scientific advances. The Department is also an open laboratory, thus foreign scientists can be visiting researchers, and it also has been designated a key lab by the Ministry of Agriculture.

Junchu Zhou, Professor and Chief of Teaching and Research for General Microbiology, described the research orientation and scope, which is mainly concerned with the basic and applied features of microorganisms useful in agriculture. The lab has 20 permanent staff, 10 research workers, 10-15 visiting scholars, 2 permanent technicians and 2 administrative staff. The priorities are : (1) molecular biology and genetic engineering of nitrogen-fixing bacteria and symbiotic systems, (2) molecular biology of Actinomycetes and genetic manipulation for production of secondary metabolites; (3) molecular biology and engineering of *Bacillus*; (4) ecological genetics of soil bacteria, and (5) multi-level interactions between microorganisms and plants.

The short-term high priority research projects are: (1) construction and application of highly efficient genetically engineered strains of soybean and peanut rhizobia (2) symbiotic genes of *Rhizobium huakuli*, (3) conservation and application of biodiversity of the Rhizobium gene pool in China, (4) study of antibiotics production and regulation; (5) improvement of antibiotics by genetic engineering, (6) exploitation of new techniques used in genetics and taxonomy of Actinomycetes, (7) isolation of the specific cry genes from Bt; (8) construction of genetically engineered Bt strains with cloned cry genes, (9) study and application of biotechnological insecticides, (10) cogenetics of plant-growth-promoting-Rhizobacteria (PGPR), (11) new methodology for *in situ* study of soil bacteria, and (12) construction and application of multi-functional genetically engineered bacterial strains.

Dr. Zhou and other scientists described in detail the work on nitrogen fixation. They have developed a high nitrogen fixing *Rhizobium japonicum* through genetic engineering, and molecular methods for characterizing Rhizobium ecology. They have a composite gene library for *Rhizobium radij* (soybeans). They have identified a protein fraction which exposes nodulation activity and can be used in a nitrogen reductase screen. The most effective isolates came from the Northern provinces of China where soybeans are grown. They have harvested several strains and plasmids, often transferring multiple genes. The strains and plasmids tested are : *R. freddi* B52 Nod fix, *R. japonicum* nif, *R. huakuii* nod and *R.leguminosarum* f *yicea* which contains sym plasmid pJB5JI. Elements transferred into each of these organisms were pJB5JI: pRA215; pRmSL26 and pMN53. All the transconjugates showed better growth and N-fixation. All were tested as inoculants in green house pot studies. They were found to be slower growing initially, but subsequently faster growing

and more effective. The research team expressed the view that potential interactions are complicated but that it is possible to improve nitrogen fixing capacity.

The main approaches to improving efficiency have included: (1) enhancing energy supply from metabolic efficiency of Rhizobia; (2) recycling of hydrogen by Rhizobium hup gene; (3) enhancing expression of BNF regulatory genes nif A or nod D; (4) increasing the copy number of BNF genes; (5) elimination of non-symbiotic plasmids; or (6) increase competitive ability of Rhizobium inoculants. They have designed a new approach for screening and testing many strains and plasmids by constructing a gene library for Rhizobia, trying triparental mating; testing in pot environment, and field experimentation in single row replicated plots with inoculations done by mixing seeds at planting.

One strain of genetically engineered rhizobia has been tested at large-scale and is nearing commercialization. In 1990 - 1992, a genetically engineered strain (HN32) of soybean rhizobia was tested in Hunan province on 10,000, 30,000 and 32,000 hectares respectively. Data from early results indicate that yields with HN32 were 2945.3 kilograms (kg) per hectare (ha), compared to 2731.5 kg/ha for a non-engineered strain of *Rhizobium japonicum* (2210), and 2522.2 kg/ha for the natural population (control). Tests are currently underway to examine the compatibility of HN32 with insecticides used on soybeans, and to assess the effectiveness of HN32 on peanuts in pot studies.

Although official permission has not yet been sought for commercialization, the recombinant rhizobia, HN32, is now being produced in a factory for use in four locations in southwestern and northeastern China. In terms of processing and consumption, soybeans harvested from experimental plots are apparently not differentiated from other soybeans.

A major research program on *Bacillus thuringensis*, (Bt), was described by **Yu Ziniu**. Three groups of 18 people are involved in this research. One project involves the isolation, characterization and identification of over 1900 Bt strains from soils all over China. Some are new strains of serotype 4. All strains are tested on different insects, and those which are most effective are given priority for further research. Several have been isolated which are effective on cotton insect pests in Shandong Province. (It was noted that some insecticides in China are no longer effective on insects, but are killing chickens which eat the resistant insects.) One insect, the Diamondback moth, is resistant to chemicals used for its control on cabbage and other vegetables. While it is showing some resistance to Bt strains, the Chinese scientists expressed confidence they can change strains of Bt and maintain control. Some Bt strains have been isolated which kill plant and animal pathogenic nematodes. Many of the currently effective strains have been isolated from western China plains and desert soils.

A second group of Bt researchers is concentrating on mass production of Bt preparations though fermentation. At present two groups of organisms are being worked on, one for forestry and the other for cotton and vegetables.

A third Bt group is concentrating on the molecular biology of Bt, led by **Chen Yahua** who has studied some in Japan. Using published sequences, he designed primers to conduct PCR and isolate *Cry* toxin genes. He has found a new strain specifically toxic to housefly larvae, called Bt "China", which is believed has only 50% homology with other Bt toxins.

Another group at the Agro-Microbiology Department, led by Qin Chongjun, is actively researching the soil microbiology of antibiotic producing actinomycetes of streptomyces species through three varying approaches. They are actively seeking ways to increase productivity of antibiotics. They have isolated an antibiotic regulatory gene and a gene to increase potency of the antibiotic in testing over 4000 isolates. They have used some strains for selective biocontrol of fungi causing rice sheath blight, corn leaf spot or cotton wilt, and claim effectiveness on root knot nematodes of tomato. Dr. Kaufman received 16 reprints covering the microbiology research described above.

A brief visit was also made to the Citrus Research Institute, in the Horticulture Department headed by **Deng Xiuxin**. A staff of 18 teaches courses for undergraduates and conducts research on heat and cold tolerant citrus species. Since 1985, 3 sosmatic hybrids have been made by protoplast fusion of mesophyll and embryogenic tissues, and regenerated into tree seedlings. Work on molecular biology and transformation is beginning.

9/28/93 Hubei Academy of Agricultural Sciences, Wuhan

Ao Li Wan, Vice President, HAAS welcomed the team. HAAS was founded in 1940, has 30 institutes, including 8 crop institutes. Many of our discussions were about the Food Crop Research Institute. HAAS has 3,500 staff, receives 2/3 of its funding from the Ministry and SSTC, and the rest from Hubei Provincial government.

Their greatest recognition has been for lean pigs (porcine growth hormone) for export to Hong Kong, and hybrid rice (HR). They have a 5-year program on collection of clonal genetic resources of tea, fruit trees, and vegetables; on a 10 hectare repository, they have a total of 7,000 entries. Biotechnology research was mostly done in the Crop Institutes (Food; Cash), in the Plant Protection Institute, and in Animal Husbandry.

Mou Tong Min, Associate Prof. in the Hybrid Rice Research Laboratory, commented on their work on 2-line hybrid rice using the Hubei PGMS. Upon recognizing Dr. Rutger, he said, "You are very famous rice scientist!" He knows Rutger's UCD student, Jinguo Hu, who is still at UCD. Prof. Mou was interested in U.S.-China cooperation on HR; there was an IRRI delegation to Beijing a few days before to discuss cooperation on HR. Prof. Mou took Dr. Rutger to see the biotechnology lab on HR. The work includes: somoclonal mutation, and protoplast fusion with Panicum and Pennisetium species for apomixis. The apomixis researcher was **He Guang Cun**. Dr. Rutger discussed various recent apomixis reports with the Chinese researchers, including the work of Chen Jiansen in Beijing.

Li Gen Jiu and **Tang Bao Yi** guided the team on a tour of the impressive Food Quality Inspection Center operated by the Ministry of Agriculture at the Academy. It had numerous analytical instruments, both U.S. and Japanese. All labs were extremely clean. There was virtually no activity at the time. Dr. Li requested assistance in making contact with USDA officials in charge of standards. He wants cooperation to help China conform to international food standards.

9/29/93 South China Agricultural University, Guangzhou

We were met by **Zhong Guilong**, Director of Foreign Affairs, who showed a 20-minute video. Regretfully, Chancellor **Lu Yong-Gen**, a former visiting scientist at UCD with Dr. Rutger, was in a meeting in Beijing. Chancellor Lu was an Associate Professor when he came to Davis; upon his return he became Chancellor.

SCAU has 1,894 staff, 23 research units, 5,000 students of whom 3,000 are undergraduates. They have many foreign ties, including foreign students.

Mei Mantong, Deputy Director of the Genetic Engineering Lab, spoke of her work on inducing useful mutants in rice, through Bevalac ion irradiation of seeds by L. Livermore National Lab in California. She obtained semidwarfs, disease resistance, etc. She also is part of the Rockefeller project on RFLP mapping of important genes in rice.

Jian Yu Yu discussed her work: (1.) protoplast fusion of indica and wild rice; (2) transformation of rice with an anti-bacterial peptide gene from silkworm, with gene gun; she just got transgenic plants recently, so has not had chance to test them for bacterial blight resistance; and (3) tissue culture of orchids for mass propagation.

Huang Zi Ran then discussed how he had isolated the anti-bacterial peptide gene from silkworm. His ultimate intent is to use this for control of bacterial diseases of humans.

Vice Chancellors **Luo Fu-He** and **Luo Shi Ming** joined us for lunch. Both were very articulate and eager to enhance cooperation with the United States. There are many opportunities for increased cooperation at this university.

9/29/93 <u>Chinese Academy of Sciences, South China Institute of Botany,</u> <u>Guangzhou</u>

Former director **Guo Jun-Yan**, who received his Ph.D. from Michigan State University in the Mid 1950s, and then returned to China, met the team. Shortly the team was joined by Director **Liang Cheng-Ye**, a 1985 Cornell graduate. SCIB has 560 staff, 8 departments, and 300 hectares. Former director Guo was an eloquent, enthusiastic talker who knows many U.S. scientists.

SCIB was established in 1929. At present it its composed of six research departments, a library, a herbarium, a beautiful botanical garden, and an arboretum. There are three ecosystem research stations located in Dinghushan, Xiaoliang and Heshan, and one center for preservation and propagation of South China rare and endangered plant species. There are about 530 staff members of which 338 are scientists and technicians. Emphasis is placed on research of plant systematics, plant taxonomy, tropical and subtropical forest ecosystems, physiology of chilling resistance, photosynthesis, post-harvest physiology of tropical and subtropical fruits, seed physiology and regulatory mechanisms, plant tissue culture and cell biology, exploitation of aromatic and medicinal plants, preservation of germplasm sources, etc.

Ling Ding-Hou, Head of the Genetics Department, discussed rice anther culture, and somaclonal induction of genetic and cytoplasmic male sterility. Dr. Rutger had met Prof. Ling 9 years ago when he was at IRRI. **Huang Yu-Wen** discussed her work on rice transformation and particle bombardment. She is trying both protoplast transformation and gene gun transformation. As in many other labs in China, she uses a homemade gene gun.

Regretfully, **Li Yuan Ching**, was on travel. However, the team learned that his current attempts on apomixis in rice are not on the twin seedlings he studied at UCD, but on a "seed set before flowering" mutant. Dr. Rutger commented that N. E. Jodon in the U.S. noted such a "seed set before flowering"

mutant (cleistogamy) about 40 years ago, but he never thought of apomixis. Former Director Guo, described work on cassava breeding.

Ding Ming Mao, microbiologist, described his work with green manure, leguminous trees, algae common to rice paddies, and mycorrhiza. This is one of several locations working on green manure, but generally farmers don't want to work with green manure in their rice paddies. They would rather produce a third rice crop.

Dr. Mao also conducts research on the Azolla (Pteridophyta, aquatic fern) and Anabaena (Cyanobacteriales) symbiotic relationship which is still used as a source on nitrogen in rice paddies. He is also working on nitrogen cycling in leguminous forest trees. His objective is to understand the carbon and nitrogen cycling in tropical and subtropical forests. This research should be helpful in China's reforestation program. Dr. Kaufman received five publications on this research.

Professor Guo showed the team the spectacular 300 hectare botanical garden, which he personally started in 1958. All and all this is an impressive group, well-equipped, but the labs virtually closed down because of upcoming holiday. There may be opportunities for increased cooperation, particularly in the areas of germplasm and biodiversity.